Supporting Information

Cytosporolides A–C, Antimicrobial Meroterpenoids with a Unique Peroxylactone Skeleton from *Cytospora* sp.

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Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with Varian Mercury -500 and -600 spectrometers using solvent signals (acetone-d_6; δ_H 2.05/δ_C 29.8, 206.1; C_6D_6; δ_H 7.16/δ_C 128.0) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0T and APEXII FT-ICR spectrometers, respectively.

Fungal Material. The culture of *Cytospora* sp. was isolated by Yang Hao from a soil sample that was collected on the Qinghai-Tibetan plateau at an altitude above 3,200 m, Linzhi, Tibet, People's Republic of China, in April, 2007. The strain was isolated from the soil suspension in distilled water by the spread-plate technique on a PDA plate with streptomycin. The isolate was identified by one of the authors (B.S.) and assigned the accession number XZ014 in X.L.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. Agar plugs were used to inoculate in Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for five days. Fermentation was carried out in six Erlenmeyer flasks (500 mL) each containing 80 g

of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Distilled H_2O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented material was extracted with EtOAc (1 L × 4), and the organic solvent was evaporated to dryness under vacuum to afford a crude extract (3.0 g). The extract was fractionated by silica gel Vacuum Liquid Chromatography (VLC) using petroleum ether–EtOAc gradient elution. The fraction eluted with 25% EtOAc (80mg) was separated by Sephadex LH-20 column chromatography (CC) using 1:1 CH_2Cl_2–MeOH as eluents. The resulting subfractions were combined and further separated by semipreparative RP HPLC (RPHPLC; Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 35% MeCN in H_2O for 17 min, followed by 100% MeCN for 20 min; 2 mL/min) to afford cytosporolide A (**1**; 8.0 mg, t_R 30.43 min). The fraction eluted with 30% EtOAc (60 mg) was purified by RP HPLC (40% MeCN in H_2O for 17 min, followed by 100% MeCN for 20 min; 2 mL/min) to afford cytosporolide B (**2**; 3.0 mg, t_R 31.02 min). The fraction eluted with 15% EtOAc (45 mg) was purified by RP HPLC (35% MeCN in H_2O for 12 min, followed by 100% MeCN for 25 min; 2 mL/min) to afford cytosporolide C (**3**; 5.0 mg, t_R 30.52 min). The fraction eluted with 40% EtOAc (200 mg) was purified by RP HPLC (30% MeCN in H_2O for 25 min; 2 mL/min) to afford fuscoatrol A (**4**; 50.0 mg, t_R 21.00 min).

Cytosporolide A (1): pale yellow oil; $[\alpha]^{25}_D$ +42 (*c* 0.2, MeOH); UV (MeOH): λ_{max} (log ε) 215 (3.62), 256 (3.22), 317 (2.85) nm; IR (neat) ν_{max} 3258 (br), 2928, 2856, 1690, 1641,

1591, 1465, 1406, 1280, 1167, 1091 cm^{-1}; ^{1}H, ^{13}C NMR, and HMBC data see Table 1; NOESY correlations (acetone-d_6, 500 MHz) H-2 ↔ H-6, H-9, H-10, H_3-13; H-3a ↔ H-12a, H_3-14; H-3b ↔ H_3-13; H-6 ↔ H-2, H-9, H_3-13; H-7a ↔ H_3-15; H-9 ↔ H-2, H-6; H-10 ↔ H-2; H-11 ↔ H-12b, OCH_3-10; H-12a ↔ H-3a; H-12b ↔ H-1, OCH_3-10; H_3-13 ↔ H-2, H-3b, H-6; H_3-14 ↔ H-3a, OH-5; H_3-15 ↔ H-7a, H-16; H-16 ↔ H_3-15, OCH_3-10, H-25b; H-19 ↔ H-25a; H-25a ↔ H-19, H-26; H-25b ↔ H-16, H-27a, H-27b; H-26 ↔ H-25a; H-27a ↔ H-16, H25b; H-27b ↔ H-25b; OH-5 ↔ H_3-14; OCH_3-10 ↔ H-11, H-12b, H-16; HRESIMS *m/z* 611.3196 $[M + Na]^+$ (calcd for $C_{33}H_{48}O_9Na$, 611.3191).

Absolute Configuration of the 5,6-Diol Moiety in 1. HPLC grade DMSO was dried with 4 Å molecular sieves. According to a published procedure,[1] a mixture of 1:1.3 diol/$Mo_2(OAc)_4$ for **1** was subjected to CD measurements at a concentration of 1.0 mg/mL. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed signs of the diagnostic bands at around 306 and 410 nm in the induced CD spectrum were correlated to the absolute configuration of the 5,6-diol moiety.

Cytosporolide B (2): pale yellow oil; $[\alpha]^{25}_{D}$ +41 (*c* 0.2, MeOH); UV (MeOH): λ_{max} (log ε) 216 (3.56), 257 (3.26), 317 (2.90) nm; IR (neat) ν_{max} 3218 (br), 2929, 2858, 1694, 1640, 1589, 1451, 1382, 1288, 1165, 1079, 1048 cm^{-1}; ^{1}H NMR (C_6D_6, 600 MHz) δ 12.68 (1H, s, OH-20), 6.49 (1H, s, H-19), 5.66 (1H, s, H-11), 4.83 (1H, d, *J* = 9.6 Hz, H-16), 4.00 (1H, d, *J* = 12.6 Hz, H-12a), 3.84 (1H, d, *J* = 12.6 Hz, H-12b), 3.65 (1H, m, H-26), 3.13 (1H, t, *J* = 10.2 Hz, H-2), 2.67 (1H, dd, *J* = 9.6, 4.2 Hz, H-9), 2.61 (1H, dd, *J* = 15.6, 4.2 Hz, H-7a), 2.38 (1H, dd, *J* = 16.0, 4.8 Hz, H-25a), 2.14 (1H, dd, *J* = 16.0, 9.0 Hz, H-25b), 2,12 (1H, m,

H-27a), 2.08 (1H, t, J = 10.2 Hz, H-3a), 1.68 (1H, dd, J = 15.6, 3.0 Hz, H-7b), 1.62 (1H, m, H-27b), 1.60 (1H, dd, J = 7.8, 3.0 Hz, H-6a), 1.59 (3H, s, H_3-24), 1.40 (3H, s, H_3-15), 1.32 (1H, t, J = 10.2 Hz, H-3b), 1.31 (2H, m, H_2-31), 1.30 (2H, m, H_2-27), 1.30 (2H, m, H_2-28), 1.30 (2H, m, H_2-29), 1.28 (2H, m, H_2-30), 1.23 (1H, dd, J = 7.8, 4.2 Hz, H-6b), 0.97 (3H, t, J = 7.2 Hz, H_3-32), 0.87 (3H, s, H_3-13), 0.68 (3H, s, H_3-14); ^{13}C NMR (C_6D_6, 150 MHz) δ 171.1 (C, C-23), 164.0 (C, C-20), 150.7 (C, C-22), 142.7 (C, C-18), 137.7 (C, C-1), 134.4 (CH, C-11), 112.0 (C, C-17), 110.1 (CH, C-19), 99.4 (C, C-21), 88.7 (C, C-8), 80.5 (C, C-5), 72.0 (CH, C-26), 67.6 (CH, C-10), 66.6 (CH_2, C-12), 63.8 (CH, C-16), 48.4 (CH, C-9), 41.7 (CH, C-2), 40.3 (C, C-4), 35.4 (CH_2, C-27), 34.6 (CH_2, C-25), 34.3 (CH_2, C-7), 34.0 (CH_2, C-3), 32.2 (CH_2, C-30), 25.9 (CH_3, C-24), 29.6 (CH_2, C-28), 28.3 (CH_2, C-6), 29.9 (CH_2, C-29), 25.1 (CH_3, C-15), 24.1 (CH_3, C-13), 23.0 (CH_2, C-31), 22.0 (CH_3, C-14), 14.3 (CH_3, C-32); HMBC data (C_6D_6, 600 MHz) H-2 → C-1, 3, 5, 11, 12; H-3a → C-1, 2, 4, 13, 14; H-3b → C-2, 4, 5, 13; H-6a → C-7, 8; H-6b → C-2, 5, 8; H-7a → C-5, 6; H-7b → C-5, 8, 9, 15; H-9 → C-7, 8, 15; H-10 → C-8; H-11 → C-2, 9, 12; H-12a → C-1, 2, 11; H-12b → C-1, 2, 11; H_3-13 → C-3, 4, 5, 14; H_3-14 → C-3, 4, 5, 13; H_3-15 → C-7, 8, 9; H-16 → C-9, 10, 17, 18, 22, 26; H-19 → C-17, 20, 21, 25; H-25a → C-18; H-25b → C-17, 18, 19, 26, 27; H-27a → C-25, 26; H_3-32 → C-30, 31; OH-20 → C-19, 20, 21; HRESIMS m/z 557.3137 $[M - H]^-$ (calcd for $C_{32}H_{45}O_8$, 557.3120).

Cytosporolide C (3): pale yellow oil; $[\alpha]^{25}_D$ +42 (c 0.2, MeOH); UV (MeOH): λ_{max} (log ε) 215 (3.58), 256 (3.34), 317 (2.96) nm; IR (neat) ν_{max} 3480 (br), 3255 (br), 2927, 2855, 1742, 1692, 1591, 1461, 1377, 1231, 1170, 1090 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.48 (1H, s, H-19), 5.78 (1H, s, H-11), 5.02 (1H, d, J = 12.6 Hz, H-12a), 4.93 (1H, d, J =

12.6 Hz, H-12b), 4.73 (1H, d, J = 9.6 Hz, H-16), 4.57 (1H, d, J = 4.4 Hz, H-10), 3.66 (1H, m, H-26), 3.47 (3H, s, OCH_3-10), 3.08 (1H, dd, J = 10.5, 8.0 Hz, H-2), 3.06 (1H, dd, J = 16.3, 4.8 Hz, H-7a), 2.94 (1H, dd, J = 15.6, 4.6 Hz, H-25a), 2.65 (1H, dd, J = 15.6, 9.3 Hz, H-25b), 2.60 (1H, dd, J = 9.7, 4.4 Hz, H-9), 2.02 (3H, s, CH_3COO), 2,10 (1H, t, J = 10.5 Hz, H-3a), 1.79 (1H, m, H-27a), 1.71 (1H, d, J = 16.3 Hz, H-7b), 1.63 (1H, m, H-27b), 1.59 (1H, dd, J = 10.5, 8.0 Hz, H-3b), 1.41 (3H, s, H_3-15), 1.32 (3H, s, H_3-24), 1.28 (2H, m, H_2-28), 1.28(2H, m, H_2-29), 1.28 (2H, m, H_2-30), 1.28 (2H, m, H_2-31), 1.17 (3H, s, H_3-13), 1.08 (3H, s, H_3-14), 0.87 (3H, t, J = 6.1 Hz, H_3-32); ^{13}C NMR (acetone-d_6, 125 MHz) δ 173.6 (C, CH_3*C*OO), 171.0 (C, C-23), 163.6 (C, C-20), 151.1 (C, C-22), 145.2 (C, C-18), 134.9 (C, C-1), 132.2 (CH, C-11), 113.5 (C, C-17), 109.4 (CH, C-19), 99.2 (C, C-21), 87.3 (C, C-8), 82.9 (C, C-5), 77.2 (CH, C-10), 72.6 (CH, C-26), 69.6 (CH, C-6), 68.2 (CH_2, C-12), 62.9 (CH, C-16), 59.2 (CH_3, OCH_3-10), 49.5 (CH, C-9), 45.7 (CH_2, C-7), 41.0 (C, C-4), 38.1 (CH, C-2), 36.6 (CH_2, C-27), 34.9 (CH_2, C-25), 34.4 (CH_2, C-3), 32.6 (CH_2, C-30), 29.6 (CH_2, C-29), 27.7 (CH_2, C-28), 26.4 (CH_3, C-24), 25.6 (CH_3, C-15), 25.3 (CH_3, C-13), 24.2 (CH_3, C-14), 23.5 (CH_3, CH_3COO), 23.3 (CH_2, C-31), 14.3 (CH_3, C-32); HRESIMS *m/z* 629.3312 $[M - H]^-$ (calcd for $C_{35}H_{49}O_{10}$, 629.3331).

Fuscoatrol A (14): ^{1}H, ^{13}C NMR, and the MS data were consistent with literature values.[2]

Antibacterial Assays. Antibacterial assays were conducted in triplicate by following the National Center for Clinical Laboratory Standards (NCCLS) recommendations.[3] The bacterial strains, *Staphylococcus aureus* (ATCC 6538) and *Streptococcus pneumoniae* (CGMCC 1.1692), were obtained from China General Microbial Culture Collection

(CGMCC) and were grown on Mueller-Hinton agar. Targeted microbes (3–4 colonies) were prepared from broth culture (37 °C for 24 h), and the final spore suspensions of bacteria (in MHB medium) were 10^6 cells/mL. Test samples (10 mg/mL as stock solution in DMSO and serial dilutions) were transferred to 96-well clear plate in triplicate, and the suspension of the test organisms was added to each well, achieving a final volume of 200 μL (antimicrobial peptide AMP was used as the positive control). After incubation (37 °C for 24 h), the absorbance at 595 nm was measured with a microplate reader (TECAN). The inhibition rate was calculated and plotted versus test concentrations to afford the IC_{50}.

References:

1. Tang, W.; Ma, S.; Yu, S.; Qu, J.; Liu, Y.; Liu, J. *J. Nat. Prod.* **2009**, *72*, 1017–1021.

2. Smetanina, O. F.; Kuznetsova, T. A.; Gerasimenko, A. V.; Kalinovsky, A. I.; Pivkin, M. V.; Dmitrenok, P. C.; Elyakov, G. B. *Russ. Chem. Bull.* **2004**, *53*, 2643–2646.

3. NCCLS 2002, NCCLS document M27-A2; NCCLS: Wayne, PA.

Figure S1. ^{1}H NMR Spectrum of Cytosporolide A (**1**; 500 MHz, Acetone-d_6)

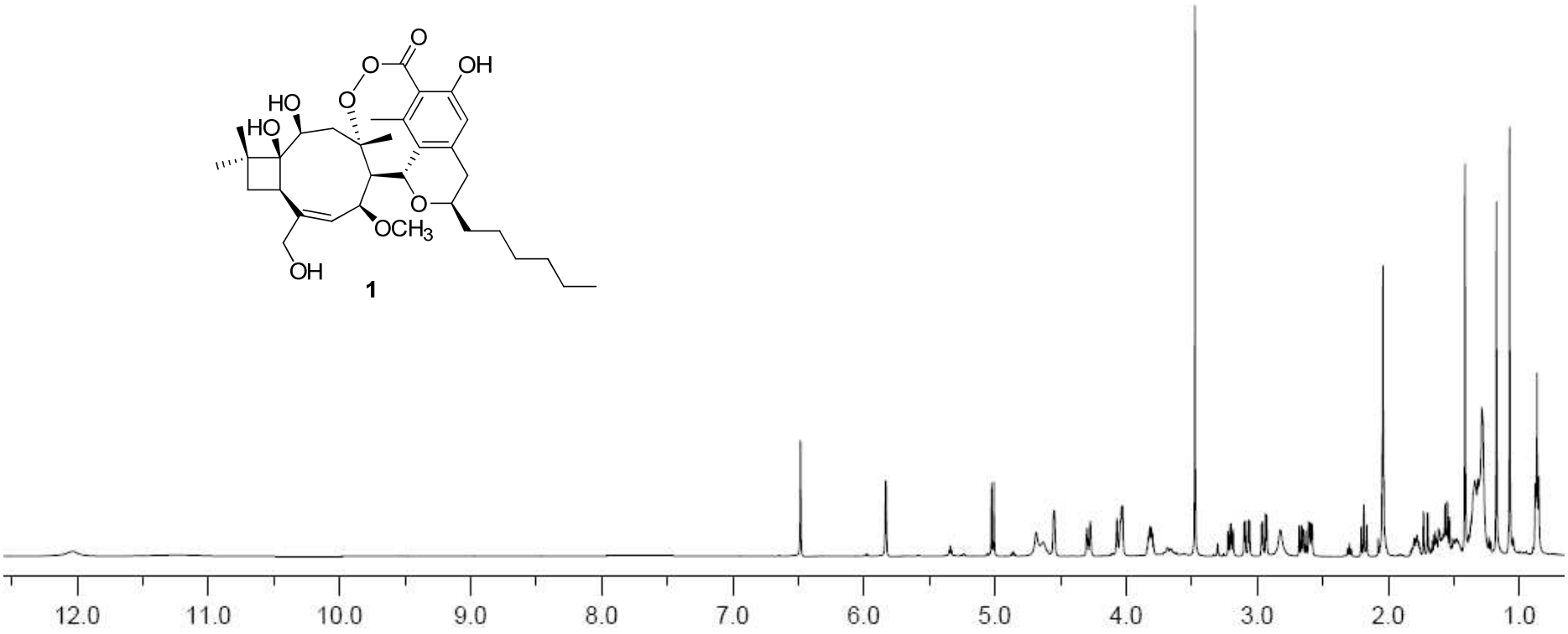

Figure S2. ^{13}C NMR Spectrum of Cytosporolide A (**1**; 125 MHz, Acetone-d_6)

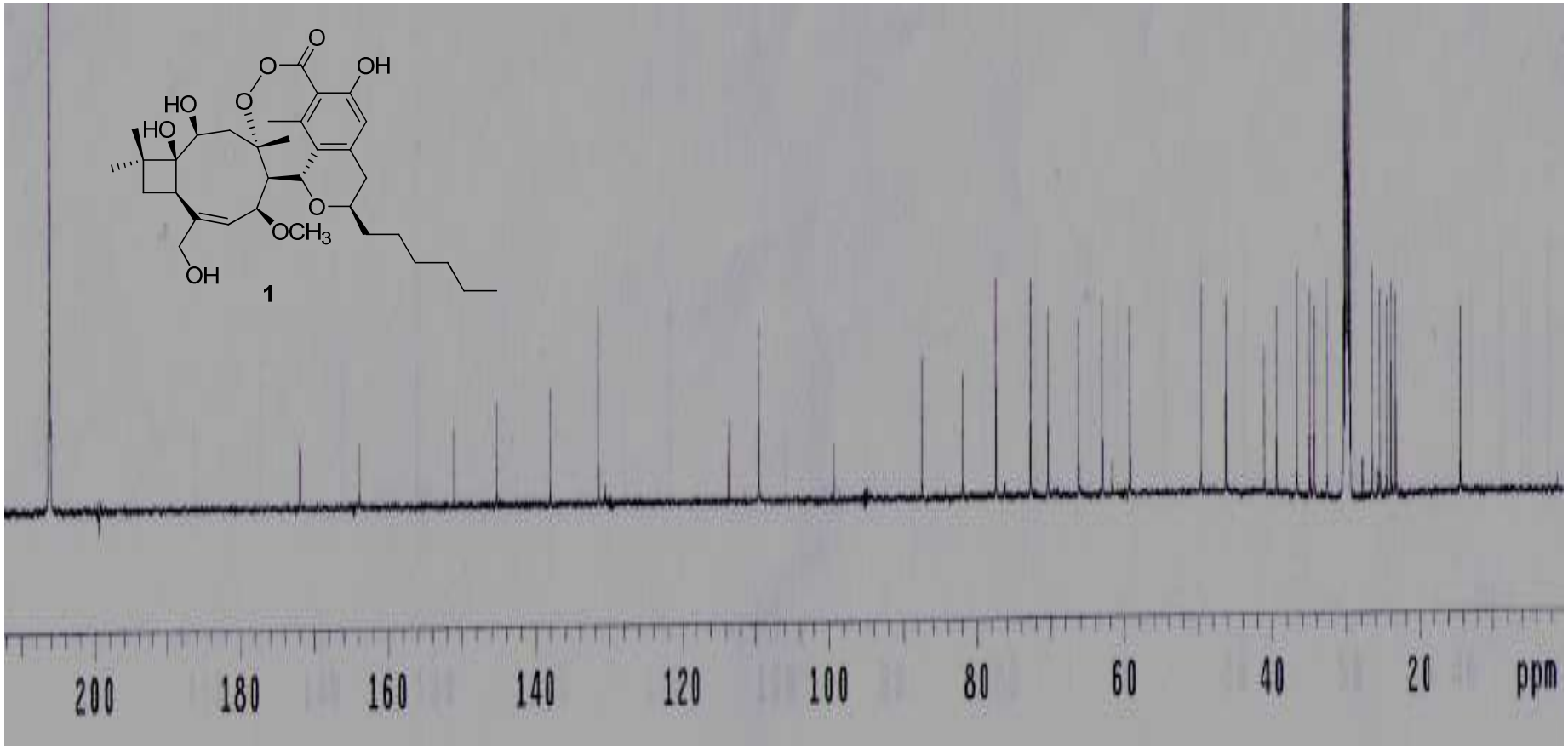

Figure S3. ^{1}H NMR Spectrum of Cytosporolide B (**2**; 600 MHz, C_6D_6)

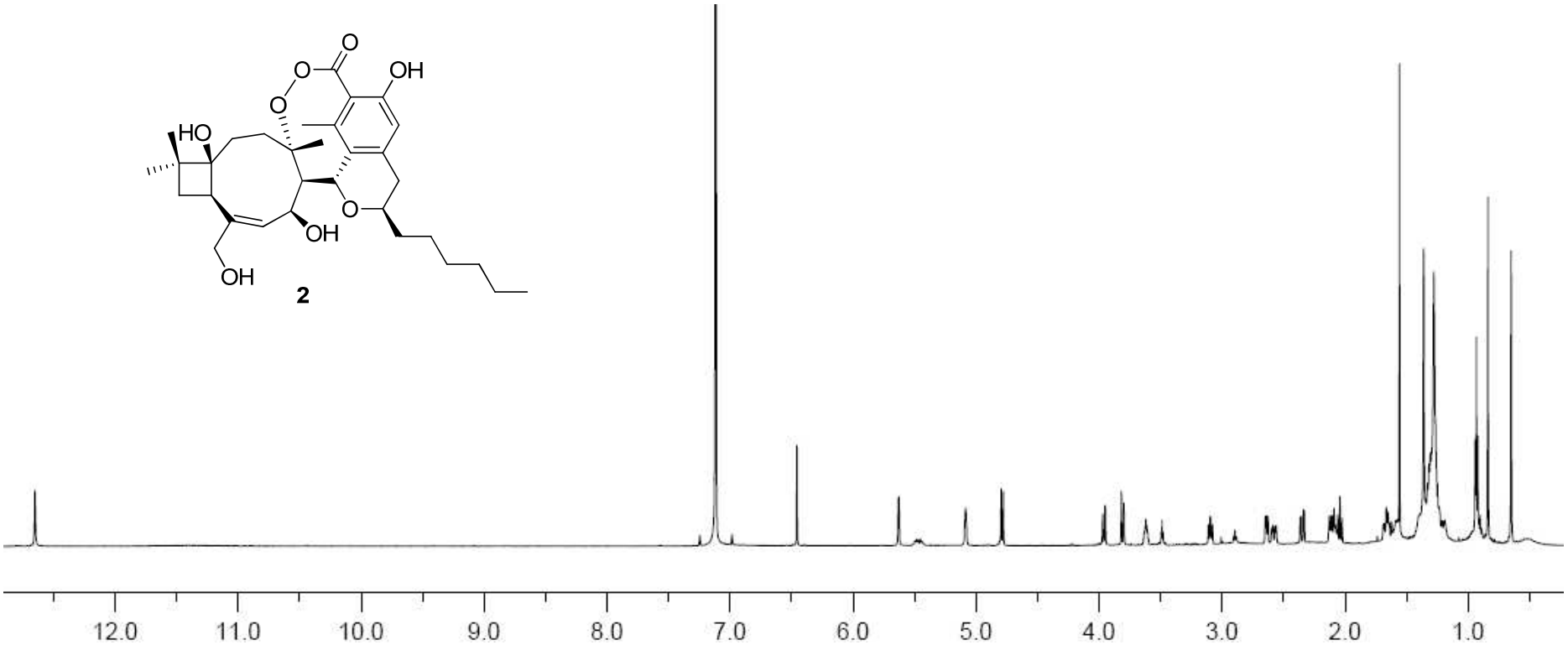

Figure S4. ^{13}C NMR Spectrum of Cytosporolide B (**2**; 150 MHz, C_6D_6)

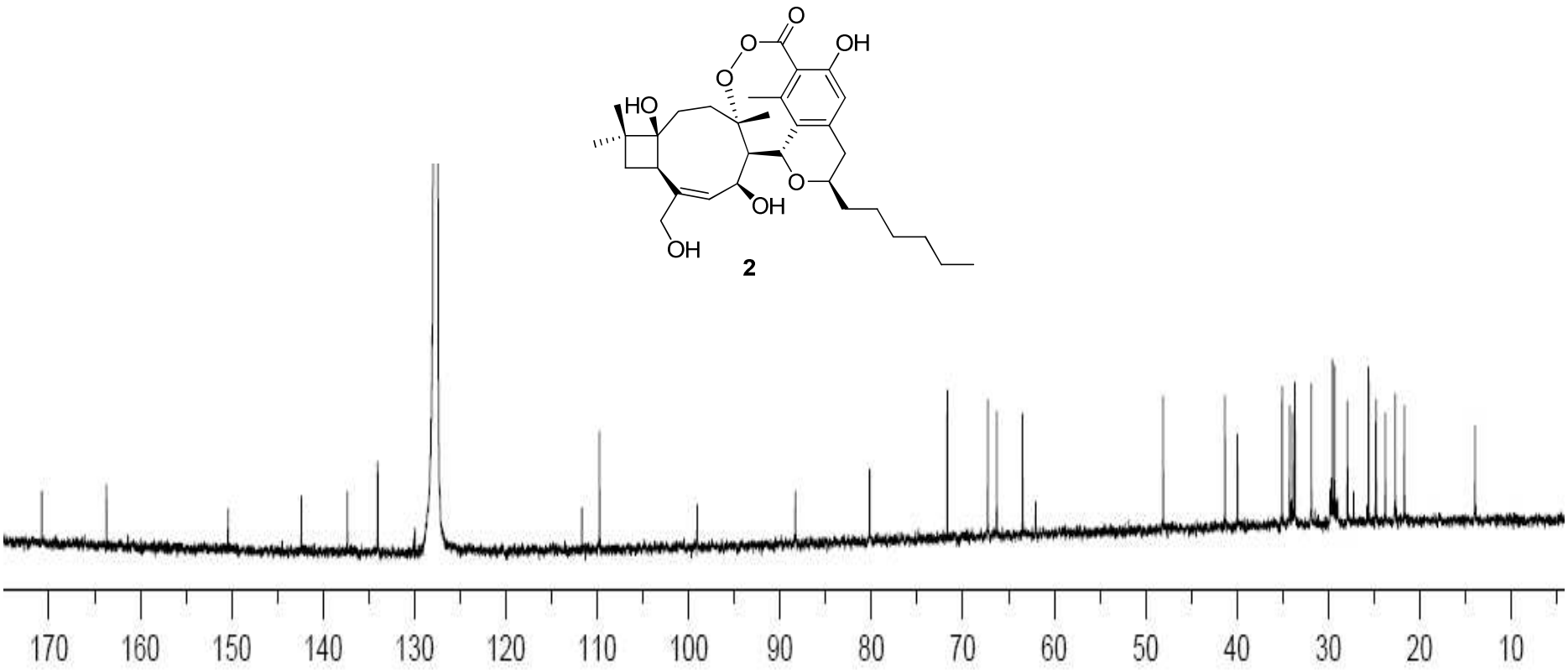

Figure S5. ^{1}H NMR Spectrum of Cytosporolide C (**3**; 500 MHz, Acetone-d_6)

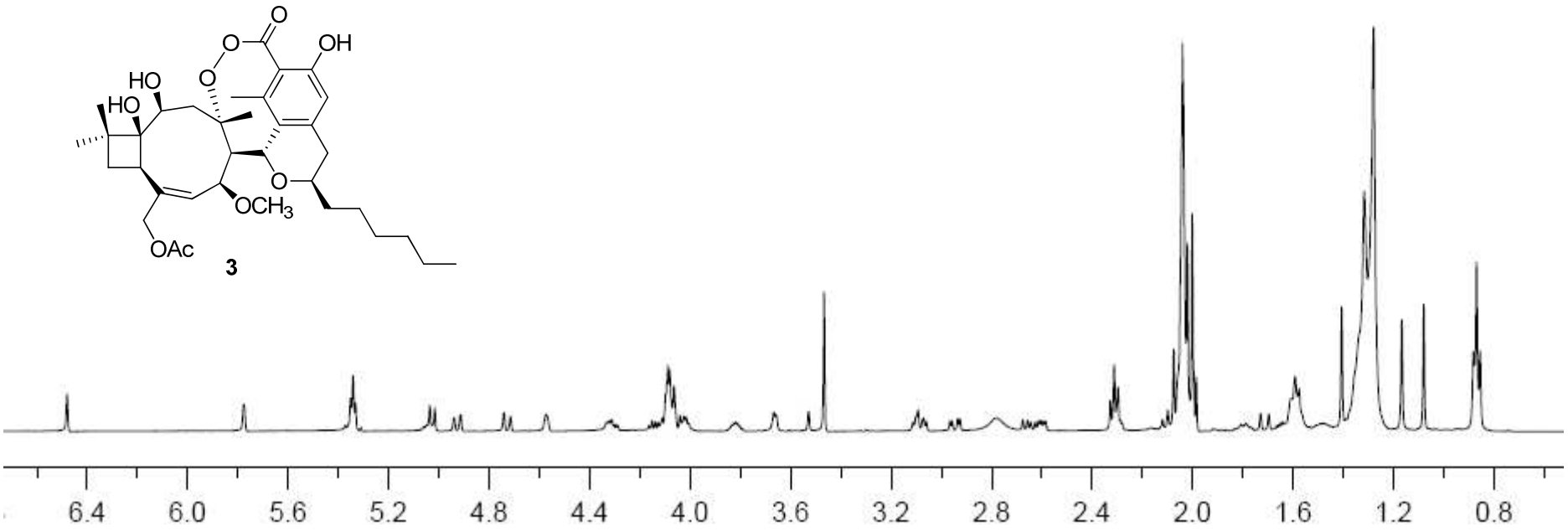

Figure S6. ^{13}C NMR Spectrum of Cytosporolide C (**3**; 125 MHz, Acetone-d_6)